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CERTIFICATE OF HAND DELIVERY

I hereby ~~certify that this~~ paper is being delivered by hand to the U.S. Patent Office in accordance with 37 CFR § 1.6(b), addressed to the Commissioner for Patents & Trademarks, Washington, D.C. 20231, on the date indicated.

Name

June 13, 2003
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of: Gregg Morin et al.

Serial No.: 09/042,460

Docket: 019/224p

Filing Date: March 16, 1998

For: MOUSE TELOMERASE REVERSE TRANSCRIPTASE

Art Unit: 1633

Examiner: Sumesh Kaushal, Ph.D.



**DECLARATION UNDER 37 CFR § 1.132**

**BY GREGG B. MORIN, Ph.D.**

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, GREGG B. MORIN, do hereby declare as follows:

1. I am a co-inventor on the above-referenced patent application, and am familiar with its contents. The claims currently under examination at the Patent Office cover nucleic acids encoding mouse telomerase reverse transcriptase (mTERT), and close homologs.

2. I understand the Patent Office has raised the question of whether someone reading the disclosure at the time it was filed would know how to make variants of mTERT that differed from the protein sequence given in the application by as much as 10%, but would still have telomerase catalytic activity when associated with telomerase RNA component.

The Patent Office has apparently taken the position that the patent disclosure describes how to make variants, but only variants that have the seven motifs shown in Figure 5 of the application.

3. I believe this position is in error, for three reasons:

- A good deal of evidence obtained before and since the filing of this application indicates that variations of over 10% of the sequence should be well tolerated
- It is known that the motifs in telomerase and in other DNA polymerase enzymes can withstand mutation without losing function
- A large number of variants could be made and screened for function in a straight-forward manner, using standard techniques, irrespective of the presence of the motifs.

4. It is clear from the sequence divergence of TERT genes that considerable variation can be tolerated without affecting function. Both the human and mouse TERT sequences are given in the application (Figure 3). These two sequences are only about 64% identical to each other at the amino acid level. Mouse TERT is less than ~30% identical to TERT proteins from other eukaryotes such as *Euplotes aediculatus* and *Saccharomyces cerevisiae.*

Yet all of these proteins perform essentially the same function of extending telomeres. In terms of the ability to bind telomerase RNA component and exhibit telomerase activity, TERT proteins appear to be interchangeable between mammals. For example, in our reconstitution experiments, we found that the RNA component from human telomerase could complex with mouse TERT to form catalytically active telomerase (Greenberg et al., Oncogene 16:1723, 1998). In another example, transfection of sheep fibroblasts with human TERT is sufficient to confer virtually limitless replication capacity (WO 02/74935, Figure 5).

It would be straightforward to make a TERT variant by substituting different amino acids into the mouse TERT sequence at the positions known to vary between different species. These variable amino acids are distributed at various positions along the full length of the TERT sequence.

5. Our experience making different types of variants confirms that the TERT amino acid sequence can be readily changed without losing telomerase function.

U.S. Patent 6,337,200 teaches functional variants of human TERT, having deletions of amino acids 192-323 or 415-450. The combined regions represent 15% of the 1132 amino acids in the intact protein. Since these entire regions can be deleted, the amino acids within these region should be amenable to considerable change without affecting function.

In unpublished work, the scientists at Geron made variants of human TERT as part of a project to assemble the protein from chemically synthesized fragments, using the technology of Gryphon Therapeutics (www.gryphonrx.com). Recombinant TERT was expressed lacking one or more of the 29 cysteines in the sequence. Most of the variants had telomerase activity when associated with telomerase RNA component.

Mouse TERT has the same general structure and function as human TERT, and should be just as resilient to changes in primary sequence.

6. The presence of motifs in a protein are useful for identifying related proteins, but are not necessarily required for function. Particular parts of a sequence may be more resistant to evolutionary change because they reside near an important region of the protein. This does not mean that the motifs are intolerant to variation.

Enclosed with this Declaration is a publication by Premal Patel and Lawrence Loeb, entitled "DNA polymerase active site is highly mutable" (Proc. Natl. Acad. Sci. 97:5095, 2000). The experiments were performed on DNA polymerase I from *Thermus aquaticus.* The polymerase contains a 13 amino acid motif ("Motif A") that is shared in polymerases from organisms separated by many million years of evolution. The motif is superimposable with a mean deviation of 1 Angstrom among mammalian pol α and prokaryotic pol I family of polymerases. The motif is homologous to what is designated as "Motif A" in the mouse TERT sequence.

They tested the plasticity of the motif by random mutagenesis. Twelve of the residues of the motif were found to be mutatable without deleting wild-type activity — as many as 10 different substitutions at some positions. One of the mutants having wild-type DNA polymerase activity had *six* amino acid substitutions within the motif.

The authors concluded that most of the motif sequence is an artifact of evolution, and had considerable plasticity relative to the basic function of the enzyme. Amongst the possible

explanations they proposed is more subtle selection pressures, or genetic transfer mechanisms operating during evolution. The same motif in mouse TERT should be equally tolerant to a wide range of variation.

7. The available evidence indicates that the evolutionary motifs present in TERT are also plastic with respect to telomerase function.

Accompanying this Declaration is an article by Tracy Bryan, Thomas Cech et al. (Proc. Natl. Acad. Sci. USA 94:8479, 1998). The article compares motif sequences in TERT from seven different eukaryotes (Fig. 1). According to the consensus residues identified in each of the motifs, variation between species was observed in 1 out of 2 residues in Motif CP; 7 out of 13 residues in Motif T; 2 out of 5 residues in Motif 1; 3 out of 5 residues in Motif 2; 7 out of 10 residues in Motif A, 9 out of 13 residues in Motif B', 4 out of 7 residues in Motif C; and 4 out of 5 residues in Motif D; and 3 out of 3 residues in Motif E.

The total variability is 42 of the 63 nucleotides in the consensus motifs (63%). They also report that when just the motif regions between human and mouse TERT are compared, they are only 76% identical (i.e., 24% different: Table 1).

The Bryan paper show variations observed in the consensus sequences in just seven naturally occurring TERT orthologs. Mutation experiments in the manner of Loeb and Patel could be used to test the full extent of plasticity of the mouse TERT sequence.

8. Someone reading this patent disclosure back at the time of filing in March of 1998 would have a range of options by which to make functional TERT variants based on the mouse TERT sequence. Mutagenesis and screening could be done on a high through-put basis without regard to the position of motifs or any other feature in the prototype sequence.

For example, restriction sites in the mTERT cDNA sequence (SEQ. ID NO:1) would be identified that flank a region to be mutagenized, and PCR primers would then be designed to span the region. Expression plasmids provided in Example 3 of the disclosure (page 103 ff.) would then be used as templates for error-prone PCR amplification under known conditions (e.g., non-optimal magnesium concentrations) that cause random mutations to be introduced. This would cause point mutations to be created anywhere within the targeted region.

Another way to introduce variation into the sequence would be to use gene shuffling, as described by W.P.C. Stemmer (Proc. Natl. Acad. Sci. USA 91:10747, 1994; and Nature 370:389,

1994). This became the MolecularBreeding™ platform technology of Maxygen Corporation (www.maxygen.com/science-platform.php), which sells commercial kits for this purpose. Briefly, the mouse TERT target sequence would be combined with homologs (e.g., human TERT, and three other TERT orthologs known at the time this patent was filed). The encoding regions are fragmented, annealed under low stringency conditions, and then amplified by PCR. This method could introduce whole segments of variant DNA into the mouse TERT sequence.

Using either of these methods to create variants, the amplified DNA would next be substituted back into the expression cassette in place of the wild-type mTERT using the restriction enzymes, and then transfected into *E. coli,* thereby producing a library of thousands of variant TERT cDNAs.

9. The TERT variant library could be easily screened for function by expressing with telomerase RNA component in a rabbit reticulocyte system (described in the patent disclosure on page 67, line 20 ff.), and then testing for telomerase function using any one of a number of possible assays (page 63, line 21 ff.). A particularly useful assay for high-throughput analysis is the dot-blot assay described on page 66.

Strategies of this kind would readily produce a large number of TERT variants. In view of the known variability of naturally occurring TERT sequences, a substantial proportion of the artificial variants would retain telomerase activity — including many that had acquired mutations in the motif regions.

10. If desired, the functional variants could then be sequenced to determine the degree of homology with the wild-type mTERT. Even more variation could be produced by subjecting the variant library to successive cycles of mutagenesis or shuffling.

Obtaining variants that differed from the prototype mTERT sequence by over 10% at the amino acid level should not pose any great difficulty.

11. I hereby declare that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

10 Jun '03
Date

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